6. SUMMARY and CONCLUSIONS

Summary

Foot ulcer infection in patients with diabetes mellitus is a major public health concern worldwide. Presently foot problems account for considerable number of diabetes related hospitalizations and moreover an important cause of non traumatic, lower-extremity amputations. It markedly decreases the quality of life, poses a huge financial burden on the patient and consumes a great deal of healthcare resources. As already discussed, multiple factors are advocated in the pathogenesis of diabetic foot ulcer particularly neuropathy and peripheral vascular disease, with infection playing an important role in the progression of ulcer. The reduction in rate of amputations can be achieved through the adoption and implementation of well-organized diabetic foot care teams, good blood sugar control and well-informed self care with relatively low investment.

Infection was found to be the major cause of amputation. The risk factors for the amputation in these patients with a diagnosis of DFU include presence of peripheral neuropathy, co-morbid conditions like nephropathy and dyslipidemia, elevated WBC, high grade of ulcer and bacterial infection type (subcutaneous and osteomyelitis) was significantly involved.

Following a diagnosis of DFU, more intensive surveillance and aggressive care by a multidisciplinary team involved in diabetic foot care may improve patient’s outcome and reduce the amputation. The result of this study, therefore, alerts us the need for proper management of the patients and in deciding the antibiotic treatment policies.

The present study, unlike in West, concludes that gram-negative bacteria dominated in DFU patients suggesting thereby that all DFU patients admitted to a tertiary care hospital in India require empirical therapy for gram positive as well as gram negative also. The treatment modes can be modified based on the severity of infection and on the microbiological culture report and current first day grams stained smear finding. The prevalence of MDR organisms is alarmingly high in the diabetic foot patients in India because of indiscriminate use of antibiotics. The findings of the present study
suggest that prospective multicentre studies are required to assess the appropriate empirical antibiotic regimen in diabetic foot ulcer infections. There is also a need for periodic antibiotic resistance surveys to help orient physicians and the local population on the best treatment strategies. The study also directs us that proper management of diabetic foot ulcers with appropriate antibiotics groups such as aminoglycoside, macrolides and chloramphenicol for *S. aureus* isolates and third and fourth generation cephalosporin’s, beta-lactum inhibitors and amino-glycoside for gram negative bacilli along with good glycemic control must be implemented, however, chloramphenicol and carbapenems can be used as a reserve drugs in infections refractory to DFU with conventional drugs. For ESBLs detection, phenotypic methods are only screening methods for detection in a routine laboratory. The genotypic methods help to confirm the genes responsible for ESBL production. Multiple genes are responsible for production of ESBLs in a single isolates as in our case. Multiplex PCR for the detection of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ gene in ESBL producing bacteria provides an efficient, rapid differentiation of ESBLs in selected species of *Enterobacteriaceae* and can be used as a rapid tool for epidemiological studies among ESBL isolates.

The observation of the present study are important, showing an association between moderate CCr (30-90 ml/min/1.73m2) and severe CCr (≤30 ml/min/1.73m2), and an increased risk for the onset of diabetic foot ulcer, poor healing and amputation. It is important to note that demonstrating an association is not the same as showing causation, which often requires an experimental design such as a randomized clinical trial and the demonstration of a common mechanism that causes CKD and failure of the skin to heal. It is likely that chronic kidney disease (CKD) and DFU among those with diabetes are associated more tightly than was recognized previously. Clinicians should be aware of the fact that diabetic patients with foot ulcers who have impaired kidney functions are at increased risk for poor wound healing and amputation even in absence of limb ischemia. Noted that, introduction of automatic reporting of eGFR each time a test for serum creatinine concentration is requested by clinician will increase the awareness of significance of kidney dysfunction in clinical practice and appropriate measures will help in improving the prognosis.
Conclusions.

The following conclusions were drawn from the study:

- Males were predominant (64.8%) compared to females (35.1%) with mean age of the diabetic foot ulcer patients as 51.1±11.4 years.

- Majority of DFU patients were having type 2 diabetes 134(82.7%).

- Presence of ulcer < 1 month was present in 63.0% patients and >1 month was 37% before presenting to the hospital.

- 124 (76.5%) of DFU patients had Ulcer size ≥4cm².

- According to the University of Texas foot ulcer grading system, 94 (58.0%) of DFU patients were from grade II, followed by grade I, 48(29.6%) and grade III 20(12.3%).

- 92(56.8%) were hypertensive (BP >140/90 mmHg) according to the ADA-2010 guidelines.

- By using the monofilament assessment methods, 72(44.4%) DFU patients had neuropathic symptoms. Retinopathy was diagnosed in 82(50.6%) DFU patients.

- Majority of the DFU patients (41.9%) were overweight (BMI 23.0-24.9kg/m²) and 16.6% were obese(BMI >25 kg/m²)

- 90.7% DFU patients had poor glycemic control (HbA1c >6.5%).

- The total cholesterol (>150mg/dl) was found in 32.7% patients. Triglycerides (>200mg/dl) in 31.5%, low HDL cholesterol (<40mg/dl) in 30.9% and increased LDL-cholesterol (>100mg/dl) in 58.0% DFU patients.

- The circulating levels of total serum protein (derange values) in 45.1% patients, serum albumin (derange values) in 57.4% and serum globulin (derange values) in 38.3% DFU patients.
• 87 (53.7%) were on oral anti-diabetic drug, 62(38.2%) on insulin and only 13(8.0%) on both at the time of admission and later on all the patients were shifted to insulin treatment.

• Previous history of amputation was found in 5.6% patients.

• Forty four percent DFU patients were smokers.

• Among the 192 diabetic foot ulcer patients, no microbial growth was observed in 15.6% patients, in the remaining 162 patients, monomicrobial infection was found in 31.4% patients and polymicrobial infection was found in 68.5% DFU patients.

• Majority (77.7%) of the cases had aerobic bacterial infection, in which 78 patients had mixed infection (with anaerobic and fungal) and 48 had monomicrobial infection. 10.4% DFU patients had anaerobic infections (mixed with aerobic infections) and 11.7% had fungal infections in which, 3 patients had pure fungal infection and remaining 16 had mixed infection (with aerobic bacteria only).

• The average number of organism isolated per patient was 1.82 in which 1.57 were aerobic bacteria, 0.10 anaerobic bacteria and 0.14 were fungal pathogens per patient.

• The most commonly isolated aerobic bacteria were *Escherichia coli* (27.8%), followed by *Staphylococcus aureus* (23.5%), *Pseudomonas aeruginosa* (15.6%), *Klebsiella oxytoca* (7.0%), *Klebsiella pneumoniae* (5.8%), *Proteus vulgaris* (3.5%), *Enterococcus faecalis* (3.5%), *Acinetobacter* sp (3.1%), Coryneform sp (2.7%), β-hemolytic streptococcus sp (2.3%), Coagulase negative *staphylococcus* (CoNS) (2.3%), *Proteus mirabilis* (1.5%) and *Morganella morganii* (0.7%).

• The most commonly isolated anaerobic bacteria was *Peptostreptococcus* sp (35.2%), followed by *Peptostreptococcus anaerobius* (23.5%), *Propionibacterium* sp (17.6%), *Bacteroides* sp (11.7%), and *Eggerthella lenta* (5.8%).

• Among the fungal pathogens, majority were *Candida* spp (75.0%) followed by *Aspergillus* spp (25.0%).
• High degree of antibiotic resistance were exhibited by *Proteus mirabilis* (67.4%) followed by *Pseudomonas aeruginosa* (67.1%), CONS (65.9%), *Acinetobacter* sp. (58.7%), *Proteus vulgaris* (55.1%), *Enterococcus faecalis* (54.0%), *Klebsiella oxytoca* (53.4%), *Klebsiella pneumoniae* (51.6%), β-hemolytic *streptococcus* (50.8%), *Staphylococcus aureus* (50.6%), *Escherichia coli* (50%), *Morganella morganii* (50%) and *Corynebacterium* sp (39.5%).

• High percentage of resistance (75.2%) was found among the Penicillin group followed by Lincosamide (71.7%), Macrolide (69.8%), Monobactams (63.5%), Aminoglycosides (61%), Quinolones & Fluoroquinolones (60.5%), Cephalosporin (57.2%), Chloramphenicol (50.8%), beta lactam inhibitors (21.7%) and Carbapenems (12.1%).

• *Staphylococcal* isolates identified as MRSA were 47(78.3%) and 41(68.3%) by using 1μg Oxacillin disc and 30μg Cefoxitin disc respectively. None of the *S aureus* were vancomycin resistant (VRSA) including those which are resistant to Oxacillin and Cefoxitin antibiotics.

• The drug resistance patterns of *Enterobacteriace* isolates showed resistance to two drugs in 11.7% isolates, three drugs in 22.6% isolates, four drugs in 3.3% isolates, five drugs in 9.2%, six drugs in 17.6% isolates, seven drugs in 5.8%, eight drugs in 7.5%, nine drugs in 8.4%, ten drugs in 6.7%, eleven drugs in 2.5% isolates whereas resistance to more than twelve or more drugs was found in 1.68% isolates.

• In the screening test result, on an average 74.2% gram negative DFU isolates were ESBL positive and 67.8% in a confirmatory test.

• Of the 66 cefotaxime resistant bacteria isolates, bla gene could be detected in 59(89.3%) using the PCR. Noting that detection of bla alleles in *E coli, Klebsiella oxytoca* and *Klebsiella pneumoniae*, we found their presence in 84.4%, 100% and 100% respectively.

• CTX-M was found to be the most prevalent ESBL noticed in 81.8%, followed by TEM in 50% isolates and SHV beta-lactamases were noticed in 46.9% isolates.
• Of the 59 bla(CTX-M+TEM+SHV) gene positive isolates, 37.2% strains were having all the three genes (CTX-M+TEM+SHV), 13.5% strains had CTX-M+SHV, 8.4% strains had CTX-M+TEM, 3.3% strains had TEM+SHV, 32.2% strains had CTX-M only, 1.6% strain had SHV only and 3.3% strain showed TEM only.

• The significant factor which are more likely to be associated with the foot ulcer were Age (40-80 years) [p<0.0003], size of ulcer >4cm² [p<0.012], site of ulcer (>1 site) [p<0.0003], hypertension [p<0.007], neuropathy [p<0.001], nephropathy [p=0.02], diabetic retinopathy [p<0.004], uncontrolled blood sugar (A1c >6.5%) [p<0.0002], higher total cholesterol (>150mg/dl) [p<0.002], triglycerides (>200mg/dl) [p<0.05], total-LDL-C (>100mg/dl) [p<0.047], lower total-HDL-C (<40mg/dl) [p<0.0006], deranged values of WBC [p<0.005], Hemoglobin [p<0.002] and RBC [p<0.0002], and higher level of SGPT/AST (>35 IU/L) [p<0.04], oral anti-diabetic treatment [p<0.005], smoking habit [p=0.03].

• The correlation of ulcer grading and the variables associated with diabetic foot ulcer. A significant correlation between University of Texas grading and duration of ulcer (p=0.014), HbA1c% (p=0.003), size of ulcer (p=0.0002), infection type [subcutaneous (p=0.047), osteomyelitis (p=0.012)], total lipid (p=0.041), total cholesterol (p=0.05), triglycerides (p=0.01), HDL-cholesterol (p=0.01), WBC (p=0.015), SGPT/AST (p=0.001), SGOT/AST (p=0.007), hypertension (p=0.041), neuropathy (p=0.004), nephropathy (p=0.024) and retinopathy (p=0.015).

• A significant correlation between Meggit Wagner Grading and HbA1c% (p=0.003), size of ulcer (p=0.0005), infection type [subcutaneous (p=0.01), osteomyelitis (p=0.015)], HDL-cholesterol (p=0.035), LDL-cholesterol (p=0.004), WBC (p=0.012), Haemoglobin (p=0.004), SGPT/AST (p=0.04), SGOT/AST (p=0.004), hypertension (p=0.015), neuropathy (p=0.014), nephropathy (p=0.015) and retinopathy (p=0.012).

• Multistep linear regression analysis was done to find out significant predictors of diabetic foot ulcer using University of Texas Grading as dependent variable and duration of ulcer (years), BMI (Kg/m²), fasting blood sugar (mg/dl), PP blood Sugar (mg/dL), HbA1c %, size of ulcer , bacterial infection type (superficial, Subcutaneous,
Osteomyelitis), T-cholesterol (mg/dl), triglycerides (mg/dL), T-HDL (mg/dL), T-LDL (mg/dL), V.L.D.L (mg/dL), TSP (gm/dL), SA (gm/dL), SG (gm/dL), SGOT/AST (I.U/L), SGPT/AST (I.U/L), ALK-phosphate (U/L), bilirubin (mg/dL), WBC (10^3/µL), Hb (g/dL), RBC, hypertensions, neuropathy, nephropathy and retinopathy.

- The factor which showed a positive association in predicting the foot ulcer by stepwise multiple linear regression analysis were duration of ulcer (years) (p=0.003), HbA1c % (p=0.004), T-HDL (mg/dL) (p=0.013), T-LDL (mg/dL) (p=0.008), SGOT/AST (I.U/L) (p=0.001), SGPT/AST (I.U/L) (p=0.007), WBC (10^3/µL) (p=0.050), hypertensions (p=0.008), neuropathy (p=0.004) and nephropathy (p=0.005).

- Significant factors which were most likely to have a risk factor for amputation were male sex [P=0.01], PVD [P=0.02], hypertension [P=0.019], chronic sensory peripheral neuropathy [P<0.002], nephropathy [P=0.02], SGPT [P<0.01], LDL-C (>100mg/dl) [P=0.014], Total cholesterol (>150mg/dl) [P<0.003], triglycerides (>200mg/dl) [P>0.005], previous antibiotic use [P<0.001], infection type such as osteomyelitis [P<0.007], subcutaneous wound infection [P=0.02] and biofilm production [P<0.0008].

- The level of CCre were significantly correlated with the status of kidney function [p<0.006], neuropathy [p=0.049], hypertension [p=0.015], grades of foot ulcer (grade 2 & grade 3) [p=0.002, p<0.001], amputation [p<0.001] and infection type (subcutaneous, osteomyelitis) [p=0.002, p<0.001].

- In a univariate analysis, odds ratio and risk ratio were calculated in between two groups of patients in which bla test were performed. The predictive factors which were associated with the bla gene (CTX-M, TEM, SHV) positivity in cefotaxime resistant Enterobacteriaceae member (E coli and Klebsiella sp) isolated from DFU patients. The significant factors which were more likely to have an association was Hospital stay (>1month) [p=0.047, OR 6.9, RR 4.67], LDL-C (>100mg/dl) [p<0.004, OR 13.4, RR 8.65] and triglycerides (>200mg/dl) [p<0.003, OR 6.5, RR 4.11] for bla gene positivity.